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L5: Entry 3 of 51 File: USPT Jun 19, 2001

US-PAT-NO: 6248570
DOCUMENT-IDENTIFIER: US 6248570 B1

TITLE: Procedures for the extraction and isolation of bacterial capsular polysaccharides for use as vaccines or linked to proteins as conjugate vaccines

DATE-ISSUED: June 19, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Michon; Francis	Bethesda	MD	N/A	N/A
Blake; Milan	Fulton	MD	N/A	N/A

US-CL-CURRENT: 435/101; 424/234.1, 424/244.1, 435/253.4, 536/123, 536/127

CLAIMS:

We claim:
1. A method of purifying capsular polysaccharides from cellular components of gram-negative and gram-positive bacteria, wherein the cellular components include protein and/or nucleic acid, the method comprising contacting the cellular components with a base reagent to obtain a mixture wherein the pH of the mixture is between about 9 and 14, separating the capsular polysaccharides from the cellular components, and recovering the capsular polysaccharides substantially free of the other cellular components.
2. The method according to claim 1 wherein a percentage of N-acetyl groups present on the capsular polysaccharide are hydrolyzed during extraction and are then re-acylated such that the re-N-acylated capsular polysaccharide is cross reactive with the native capsular polysaccharide.
3. The method of extracting capsular polysaccharides from cellular components of gram-negative and gram-positive bacteria according to claim 1 further comprising the steps:
(a) separating the capsular polysaccharide from the other cellular components by chromatography;
(b) reacting the capsular polysaccharide from step (a) with an acylating agent;
(c) purifying the capsular polysaccharide from step (b) by chromatography.
4. The method according to claim 1, wherein the pH of the mixture is about 12.
5. The method according to claim 3, wherein the capsular

polysaccharide is derived from any bacterium of the genus Streptococci.
6. The method according to claim 3, wherein the capsular polysaccharide is derived from group B Streptococci.
7. The method according to claim 3, wherein the capsular polysaccharide is derived from group B Streptococci types Ia, Ib, II, III V, or VI or VIII.
8. The method according to claim 3, wherein the base reagent comprises an organic base.
9. The method according to claim 3, wherein the base reagent comprises an inorganic base.
10. The method according to claim 3, wherein the base reagent comprises NaOH, KOH or LiOH.
11. The method according to claim 3, wherein the separating by chromatography step is hydrophobic-interaction chromatography.
12. The method according to claim 3, wherein the acylating agent is acetic anhydride, acetyl chloride, pentafluorophenyl acetate or 4-nitrophenyl acetate.
13. The method according to claim 3, wherein the purifying the capsular polysaccharide by chromatography step is gel-permeation chromatography.
14. The method according to claim 3, wherein the base reagent comprises an inorganic base, the separating by chromatography step is hydrophobic chromatography, the acylation reagent is acetic anhydride, acetyl chloride, pentafluorophenyl acetate or 4-nitrophenyl acetate, and the purifying the capsular polysaccharide by chromatography step is gel-permeation chromatography.
15. The method according to claim 3, wherein the base reagent comprises NaOH, hydrophobic-interaction chromatography is used in step (b) to separate capsular polysaccharide from nucleic acid, the acylating agent is acetic anhydride and the capsular polysaccharide is recovered in step (c) by gel filtration chromatogaphy.
16. The method according to claim 3, wherein the capsular polysaccharide is derived from any bacterium of the genus Neisseria.
17. The method according to claim 3, wherein the capsular polysaccharide is derived from N. meningitidis type C.
18. The method according to claim 1, wherein the purified capsular polysaccharide contains less than about 1% by mass of nucleic acid and less than about 1 ug/ml protein.
19. A method of purifying capsular polysaccharide from cellular components including nucleic acid and/or protein of gram-negative and gram-positive bacteria, the method comprising contacting bacterial cells, homogenized bacterial cells, bacterial culture supernatant, or a mixture thereof with a base reagent to obtain basic conditions sufficient to hydrolyze base labile bonds, separating the capsular polysaccharide from the other cellular components, and recovering the capsular polysaccharide substantially free of the other cellular components.
20. The method according to claim 19, wherein the basic conditions are between about pH 9 and pH 14.
21. The method according to claim 19, wherein the capsular polysaccharide contains N-acetyl groups and wherein at least a portion of these N-acetyl groups are hydrolyzed by treatment

with the base reagent.
22. The method according to claim 20, wherein the basic conditions are about pH 12.
23. The method according to claim 22, wherein the method comprises contacting bacterial cells with the base reagent.
24. The method according to claim 19, wherein the purified capsular polysaccharide contains less than about 1% by mass of nucleic acid and less than about 1 ug/ml protein.
25. The method according to claim 19, wherein the separating step is chromatographic separation.
26. The method according to claim 19, wherein the capsular polysaccharide is derived from any bacterium of the genus Neisseria.
27. The method according to claim 26, wherein the capsular polysaccharide is derived from N. meningitidis type C.
28. The method according to claim 19, wherein the capsular polysaccharide is derived from any bacterium of the genus Streptococci.
29. The method according to claim 28, wherein the capsular polysaccharide is derived from group B Streptococci.
30. The method according to claim 29, wherein the bacteria are group B Streptococci types Ia, Ib, II, III V, VI or VIII.
31. A method of producing a group C meningococcal polysaccharide conjugate vaccine comprising (a) contacting group C meningococcal bacterial cells, homogenized bacterial cells, bacterial culture supernatant, or a mixture thereof comprising a group C meningococcal capsular polysaccharide with a base reagent to obtain basic conditions sufficiently basic to hydrolyze at least one N-acetyl group of the group C meningococcal polysaccharide, (b) separating the capsular polysaccharide from the product of step (a) and conjugating the polysaccharide to a polypeptide.
32. The method according to claim 31, wherein conjugation is accomplished by reductive amination.
33. The method according to claim 32, further comprising the steps of treating the de-N-acetylated polysaccharide with an acylating agent and treating the N-acylated polysaccharide with an oxidizing agent to oxidatively cleave vicinal diols to produce aldehyde groups.
34. The method according to claim 33, further comprising isolating the de-N-acetylated polysaccharide and isolating the re-acylated product.
35. The method according to claim 33, wherein the base reagent is selected from the group consisting of sodium hydroxide, potassium hydroxide and lithium hydroxide and the acylating agent is selected from the group consisting of acetic anhydride and acetyl chloride.
36. The method according to claim 1, wherein the pH of the mixture is sufficient to degrade nucleic acid.
37. The method according to claim 19 or 31, wherein the basic conditions are sufficient to degrade nucleic acid.
38. The method according to claim 1, 19, or 31, wherein the method is protease free.
39. The method according to claim 1, 19, or 31 wherein the method is nuclease free.
40. The method according to claim 1, wherein the method comprises contacting bacterial cells, homogenized bacterial

cells, bacterial culture supernatant, or a mixture thereof with the base reagent.

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Michon; Francis	Bethesda	MD	N/A	N/A
Blake; Milan	Fulton	MD	N/A	N/A

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
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APPL-NO: 9/ 221630
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PARENT-CASE:
Priority is claimed from U.S. Provisional Application Ser. No. 60/068,608 filed Dec. 23, 1997 which is incorporated herein by reference.

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US-CL-CURRENT: 435/101; 424/234.1, 424/244.1, 435/253.4, 536/123, 536/127
FIELD-OF-SEARCH: 435/101, 435/253.4, 536/127, 536/123, 424/244.1, 424/234.1, 424/250.1

PRIOR-ART-DISCLOSED:

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Search Selected Search ALL

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☐	4356170	October 1982	Jennings et al.	424/92
☐	4413057	November 1983	Carlo et al.	N/A
☐	5190746	March 1993	Cassels et al.	N/A

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ART-UNIT: 161
PRIMARY-EXAMINER: Prats; Francisco
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ABSTRACT:

A procedure to isolate large quantities of capsular poly saccharides (CPS) from culture supernatants as well as bacterial cells of gram-negative and gram-positive bacteria

using base extraction is described. The procedure is simple, rapid, reproducible and applicable to a variety of bacterial species. The method also yields novel CPS characterized by their lack of covalent attachment to extraneous peptidoglycan. Vaccines and methods of immunization against bacterial infection using the CPS obtained by the process of the invention are also disclosed.

40 Claims, 12 Drawing figures